DISCUSSION OF THE AMENDMENT

The specification has been amended as shown in the Substitute Specification submitted herewith. No new matter is believed to have been added thereby.

All the claims have been cancelled and replaced with new Claims 9-32. Claim 9 is supported by Claim 1, combined with symptoms (1) and (2) of Claim 2, and Example 3. Claim 15 is supported by Claim 1, combined with symptom (2) of Claim 2. Claim 21 is supported by Claim 1 and symptom (3) of Claim 2. Claim 27 is supported by Claim 1 and symptom (4) of Claim 1. The dependent claims on each of new Claims 9, 15, 21, and 27, correspond to original Claims 3-7, respectively.

No new matter is believed to have been added by the above amendment. Claims 9-32 are now pending in the application.

REMARKS

The rejection of Claims 1-8 under 35 U.S.C. § 103(a) as unpatentable over JP8-165248 (JP '248) taken with the article by Elass-Rochard et al in *Infection and Immunity*, Vol. 66, No. 2, pp. 486-491, February 1998 (Elass-Rochard et al), is respectfully traversed.

The presently-claimed invention is now drawn to a method for alleviating a symptom from lipopolysaccharide-induced inflammation comprising administering to a person orally or parenterally an effective amount of human-type lactoferrin for a time and under conditions effective to alleviate said symptom. The symptom is accumulation of body fluid containing albumin at the inflammation site, as recited in Claim 9; accumulation of albumin at the inflammatory site, as recited in Claim 15; decrease of albumin concentration in blood, as recited in Claim 21; or increase of neutrophils in blood, as recited in Claim 27.

As described in the specification at paragraph [0003], lactoferrin (Lf) has been demonstrated in vitro to form a chelate with iron to mainly inhibit growth of E. coli, etc., and shows a bactericidal effect, as well as other pharmacological effects. As disclosed in paragraph [0004] of the specification, on the other hand, in inflammatory diseases, for example, ascites in peritonitis and bronchocavernous plasma exudation in pneumonitis are retained respectively to cause a decrease of physical strength of patients. Also severe exudation of neutrophils and tissue damage are induced in the inflammatory site. In sepsis caused by gram-negative bacilli, it is known that decline in blood albumin concentration, decrease of lymphocytic leukocytes, and increase of neutrophils occur and sometimes induce deterioration of the symptom, which in turn is developed into a systemic inflammatory reactive syndrome such as multiple organ failure, of which the prognosis is quite worse. In order to improve these symptoms resulting from inflammation, it has been attempted to administer a human-type albumin preparation into blood, but no sufficient alleviation effect for the symptoms has been recognized. In a case of serious invasion such as injury or a

highly inflammatory state such as severe infection, it is considered that a large quantity of plasma water, would exude into the tissue because of sthenia of the endothelial permeability. In such a case, it has been reported that the use of a colloidal material such as an albumin preparation to alleviate the symptom has unexpectedly increased the risk of death. There is accordingly a demand for the development of a new agent to effectively alleviate these symptoms.

Thus, when inflammation occurs, endothelial permeability becomes high. Therefore, albumin in the blood exudes out of the blood vessel to the inflammatory site or region. Naturally, serum water also exudes and accumulates at the inflammatory site to cause swelling, edema, accumulation of ascites, etc. For example, in the case of arthritis, accumulation of body fluid in the diseased knees causes pain.

The above-described albumin-exudation causes hypoalbuminemia that brings about lowering of immunity. Transfusion of an albumin preparation is employed as an expectant treatment for hypoalbuminemia. However, administered albumin exudes out of the blood vessel in an advanced state of endothelial permeability. In addition, transfusion of an albumin preparation is troublesome and is attended by risks of infection.

The present invention contributes to relief of pain in lipopolysaccharide (LPS)-induced inflammation and/or contributes to the prevention or remediation of hypoalbuminemia by suppressing exudation of albumin out of the blood vessel.

JP '248 discloses the use of Lf and peptides thereof for preventing release of the endotoxin-inducing cytokine from an immune-system cell, for example, release of interleukin-6 by the endotoxin stimulation from a human monocite, and is thus effective as a suppressing agent of inflammation by the endotoxin. However, JP '248 discloses and suggests nothing regarding albumin exudation induced by LPS, and thus, JP '248 neither discloses nor suggests a method for alleviating (1) accumulation of body fluid containing

albumin at the inflammatory site, (2) accumulation of albumin at the inflammatory site, or (3) decrease of albumin concentration in blood, by using human lactoferrin (hLf). Nor does JP '248 disclose or suggest anything with regard to alleviating (4) increase of blood neutrophils by using hLf.

Nor is the Examiner's finding with regard to JP '248's description of doses correct. Rather, the dosage range(s) therein are **not** for hLf but for the **peptide** derived from bovine Lf and the synthetic **peptide** corresponding thereto. In addition, no disclosure in JP '248 can be found that supports a range of 1 mg/kg to 50 mg/kg.

Elass-Rochard et al does not remedy the above-discussed deficiencies in JP '248.

Elass-Rochard et al discloses that "[t]he ability of hLF to form complexes with LPS (3, 11) and thus to inhibit the LPS-induced release of cytokines by mono-nuclear phagocytes (7, 27) makes it a potentially important molecule in the inflammatory response" (page 489, under "Discussion.") Specifically, Elass-Rochard et al discloses that hLF prevented the rhLBP-mediated binding of LPS to the CD14 receptors on the cells. "Maximal inhibition of LPS-cell interactions by hLF was raised when both hLf and rhLBP were simultaneously added to LPS or when hLf and LPS were mixed with cells 30 min. prior to the incubation with rhLBP" (page 486, under "Abstract.")

The albumin accumulation in the rats to which hLf was injected 18 hours prior to the administration of LPS was less than that in the counterparts of the rats to which hLf was injected only 15 minutes prior to the administration of LPS, as shown in Example 4 in the specification herein. See present Fig. 6. Accordingly, the competitive inhibition shown in Elass-Rochard et al cannot explain the present invention.

Further, it should be noted that the tests reported in Elass-Rochard et al were carried out *in vitro*, and that mechanisms concerning inflammation in the living body are very

complicated, as seen, for example, from the following description at the end of Elass-Rochard:

Further in vivo studies are needed to investigate whether Lf could directly overcome the LBP-mediated activation of cells in the host and modulate the CD14-independent LPS signaling pathways.

In response to the Examiner's finding at page 7 of the Office Action that Elass-Rochard et al at page 486, first paragraph, right column, "continues by stating that *in vivo*, hLf also regulates the release of TNF-α and protects mice against a lethal dose of *E. coli*. Thus, clearly showing that use of hLf increases blood neutrophils and generates TNF-α, and as such meet the limitation of claim 2," on the contrary, the use of hLf in the present invention alleviates abnormal increase of blood neutrophils induced by LPS injection as shown in Example 1 in the specification herein. See present Fig. 2, wherein the blood was collected from the abdominal aortas as disclosed therein.

In sum, Elass-Rochard et al discloses and suggests nothing with regard to albumin exudation or increase of blood neutrophils, and thus nothing with regard to the presently-claimed invention.

In response to the Examiner's findings in the paragraph bridging pages 8 and 9 of the Office Action, the specification herein does **not** describe that bovine Lf has been used to demonstrate an effect of alleviating accumulation of body fluid containing albumin, decrease of blood albumin concentration or increase of blood neutrophils.

It is known that in the case of sepsis caused by gram-negative bacilli, blood albumin concentration decreases and blood neutrophils increases. However, at the time of the present invention, there was no report disclosing (1) that human or bovine Lf can suppress accumulation of body fluid containing albumin, decrease of blood albumin concentration or increase of blood neutrophils, or (2) that IL-6 brings about decrease of blood albumin concentration or increase of blood neutrophils.

Thus, the present invention, directed to a method for alleviating by using hLf, (1) accumulation of body fluid containing albumin at the inflammatory site, (2) accumulation of albumin at the inflammatory site, (3) decrease of blood albumin concentration in blood, or (4) increase of neutrophils in blood is not suggested by either JP '248 or Elass-Rochard et al, or their combination.

For all the above reasons, it is respectfully requested that the rejection over prior art be withdrawn.

The rejection of Claims 1-8 under 35 U.S.C. § 112, first paragraph, is respectfully traversed. The claims are now limited to LPS-induced inflammation. Thus, the rejection is now moot. Accordingly, it is respectfully requested that it be withdrawn.

The rejection of Claims 1-8 under 35 U.S.C. § 112, second paragraph, is respectfully traversed. The Examiner finds that "[i]t is not clear what is the difference between 'body fluid' and 'albumin' in a Markush format claim. It appears to be genus and species situation." In reply, body fluid is a liquid. Albumin is a component of the blood. Plasma water that contains albumin exudes from the blood vessel to the inflammation site and accumulates to form pools of body fluids like ascites. The volume of accumulated ascites is not directly proportionate to the concentration of albumin. Therefore, body fluid and albumin, respectively, are not in a "genus" and "species" relationship.

The rejection is otherwise moot. Accordingly, it is respectfully requested that it be withdrawn.

The objection to the specification is now moot in view of the Substitute Specification submitted herewith. Accordingly, it is respectfully requested that the objection be withdrawn.

All of the presently-pending claims in this application are now believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

Respectfully submitted,

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